Effect of anaerobic digestion at 35, 55 and 60 °C ON pharmaceuticals and organic pollutants

Davidsson, Å.; Kjerstadius, H.; Haghighatafshar, S.; Fick, J. ; Eriksson, Eva; Olsson, Mikael Emil; Wachtmeister, H.; Cour Jansen, J. la

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

EFFECT OF ANAEROBIC DIGESTION AT 35, 55 AND 60°C ON PHARMACEUTICALS AND ORGANIC CONTAMINANTS

Å. Davidsson[1], H. Kjerstadius[1], S. Haghighatafshar[1], J. Fick[2], M. Olsson[3], H. Wachtmeister[3], E. Eriksson[3], J. la Cour Jansen[1]

[1]*Water and Environmental Engineering, Department of Chemical Engineering, Lund University, P.O. Box 124, 221 00 Lund, Sweden*

[2]*Department of Chemistry, Umeå University, 901 87 Umeå, Sweden*

[3]*Department of Environmental Engineering, Technical University of Denmark, Miljoevej 113, DK-2800 Kgs. Lyngby, Denmark*

E-mail of the corresponding author: asa.davidsson@chemeng.lth.se

Abstract

The application of treated sewage sludge on farmland is a suggested method for recycling nutrients and reducing demand for commercial fertilizer. However sludge needs to be rendered safe from possible contaminants which can cause acute and long-term health and environmental problems. Residual pharmaceuticals and organic contaminants in sludge are mentioned as emerging threats since wastewater treatment plants are not designed to degrade these substances thus yielding an accumulation in sludge. The aim of this study was to evaluate the presence, and reduction, of pharmaceuticals and polycyclic aromatic hydrocarbons (PAHs) during anaerobic digestion at 35, 55 and 60ºC and during pasteurization at 70°C. The substrate used was mixed primary and secondary sludge from a 300 000 person-equivalents municipal wastewater treatment plant in southern Sweden. In general no reduction of pharmaceuticals could be observed at any temperature or minimum exposure time, except for the beta-blocker Irbesartan and the antibiotic Trimethoprim. The results from pharmaceuticals in mesophilic sludge agreed with results in recent Swedish studies. Also, no reduction of PAHs during digestion or pasteurization (70°C – 1 hour) was seen, but for single PAHs digestion could lead to reduction.

Keywords: Anaerobic digestion, organic contaminants, pharmaceuticals, sludge

INTRODUCTION

To be able to apply treated sewage sludge on farmland for recycling of nutrients and reducing demand for commercial fertilizer the sludge need to be rendered safe from possible contaminants which can cause health and environmental problems. Pathogens, residual pharmaceuticals and organic contaminants in sludge are mentioned as emerging threats since

wastewater treatment plants (WWTPs) are not designed to degrade or mineralise these substances thus yielding an accumulation in sludge (Naturvårdsverket, 2008).

Polycyclic aromatic hydrocarbons (PAH) and polychlorinated bi-phenyls (PCB) are micropollutants with known toxic and carcinogenic properties both for humans and aquatic organisms (Økland et al., 2005). The groups include some hardly degradable compounds with long degradation times (Naturvårdsverket, 2003; Økland et al., 2005). The proposal for a new EU Water Framework Directive (WFD) (EC, 2012) is mostly focused on the PAHs since the use of PCBs is banned and the PCB concentrations in sludge has been seen to decrease the latest decades in both EU and other parts of the world (Clarke et al., 2008; Clarke et al., 2010; Erhardt & Prüeß, 2001). The studies by Clarke et al. (2008, 2010) also showed that the degradation is increased with a higher temperature and that some PAHs were more efficiently removed at thermophilic digestion. In Trably et al. (2003) it was shown that the removal efficiency correlated with the methanogenic activity.

Environmental effects from pharmaceutical residues in wastewater are of great interest since humans excrete pharmaceuticals and their metabolites, which can possibly end up in the environment with the treated wastewater (to recipients) or as sludge being used as fertilizer on agricultural land. Effects on aquatic organisms in recipients have been seen from endocrine disruptors and eventual increase of resistance to antibiotics on bacteria caused by residues in wastewater effluents or re-used sludge is discussed at the moment. No statutory limit concentrations for pharmaceutical residues in effluent wastewater or stabilised sludge from WWTPs exist, but the proposal for a new EU WFD suggest to keep track of Diclofenac, Ethinylestradiol and Estradiol (EC, 2012).

There are two possibilities to make the sludge less harmful for the environment and possible to re-use as fertiliser: 1) the contaminants can be avoided in the original wastewater by up-stream work (mitigation near the source of the pollution e.g. by reducing industrial discharges, local treatment at hospitals and by educating inhabitants) and 2) the contaminants could possibly be reduced/degraded in the sludge treatment processes at the WWTP, such as the anaerobic digestion or, if occurring, a separate hygienization step or other pre/post-treatment methods. This study has focused on finding out the potential of the second possibility by evaluating the presence and reduction of 99 pharmaceuticals and 15 PAHs in sludge during anaerobic digestion at 35, 55 and 60ºC. These analyses were made in connection to measuring the pathogen reduction after continuous digestion at different temperatures with different minimum exposure times (time between feeding). Included in the study was also a separate hygienization step in form of pasteurization at 70°C for 1 hour, which has been suggested as a means of reducing pathogens in sludge; more details are found in Kjerstadius et al. (2013). The substrate used was mixed primary and waste activated sludge from a medium-sized 300 000 person-equivalents municipal WWTP in southern Sweden.

METHODS

Sludge consisting of a mix of primary sludge (25%) and biological sludge (75%) from the Sjölunda Wastewater treatment plant in Malmö, Sweden was pasteurized at 70°C for 1 hour or digested in anaerobic reactors.

The anaerobic digestion test setup was a series of 20 L semi-continuously fed stirred-tank reactors (CSTR's), each set to a combination of temperatures (35, 55 and 60ºC) and hydraulic retention times (7 and 15 days). At steady-state conditions and after minimum exposure times

of 2, 2.5, 6 and 24 hours samples were removed and analyzed for concentration of the pharmaceutical substances and PAHs. Minimum exposure time is here defined as the time between feeding of new sludge and withdrawal of digested residue used when operating the semi-continuously fed digesters. Pasteurization of sludge was done by heating up raw sludge during 30 min in water bath and then keeping it at 70ºC for 1 hour. Total solids (TS) and volatile solids (VS) were determined according to DS/EN 872:1997.

Pharmaceutical residues

All sludges were freeze-dried and 0.1 g (dry weight) sample aliquots were used for extraction, to which internal and surrogate standards were added before extraction. Sequential extraction was performed using ethyl acetate and methanol (1:1) followed by methanol and water (7:3) with 5% triethylamine. Samples were homogenized for four minutes, at 42000 oscillations per minute, using a Mini Beadbeater (Biospec. Bartlesville, USA) with zirconium beads and then centrifuged at 14000 rpm for 10 min. This protocol was done for both eluent mixtures and the supernatants were combined, evaporated to 20 µL and reconstituted in 1 ml water and acetonitrile (95:5 mixture) with 0.1% formic acid.

All pharmaceuticals were analyzed with the same methodology as reported in Grabic et al. (2012). In short, a triple stage quadrupole MS/MS TSQ Quantum Ultra EMR (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland) were used as analytical system. Twenty µL of the sample was loaded onto a Hypersil GOLD aQ TM column (50 mm x 2.1 mm ID x 5 µm particles, Thermo Fisher Scientific, San Jose, CA, USA) preceded by a guard column (2 mm×2.1 mm i.d, 5 µm particles) of the same packing material and from the same manufacturer. Both heated electrospray (HESI) and atmospheric pressure photoionization (APPI) in positive and negative ion modes were used for ionization of target compounds. The same method was used to investigate the fate of APIs in wastewater treatment by Hörsing et al. (2011) and Hey et al. (2012) and a full method evaluation and detailed description is given in Grabic et al. (2012).

Polycyclic aromatic hydrocarbons

The sludge samples (digested, pasteurized and untreated sludge from the four test rounds with minimum exposure times 24h, 6h, 2h and 2.5h) were freeze-dried and then stored in 4°C until analysis. Microwave assisted extraction of 0.5 g freeze-dried sludge was performed in a Multiwave 3000SOLV, Anton-Paar using hexane and acetone (3:2). Thereafter the extracts were filtrated through glass wool and 2 ml isooctane (2,2,4-trimethylpentane), containing 100 µg/l naphthalene-d8, pyrene-d10 and phenantrene-d10 as internal standards were added before evaporation to 2 ml (with N_2 or compressed air). Conditioned solid phase (SPE) columns (LC-Florisil) were used for concentration and clean-up and the analytes were eluted by hexane and toluene (4:1). SPE-extracts were evaporated to 2 ml and transferred to GC-MS vials. GC-MS was used for separation and detection of all PAHs simultaneously in each sample. 15 of the US EPA list of 16 PAHs were determined (acenaphthene, acenaphtylene, anthracene, benzo[a]antracene, benzo[a]Pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]perylene, naphthalene, phenanthrene and pyrene).

RESULTS AND DISCUSSION

Pharmaceutical residues

In total 99 pharmaceutical substances were analysed (in duplicates or triplicates) in sludge samples (untreated, pasteurized (1h - 70°C) and digested at 35, 55, 60°C with different minimum exposure times). Some (72) of the substances were found in at least one sludge sample, but only 19 of the substances were found in both untreated, pasteurized and digested sludge. Average measured concentrations of chosen substances are presented in Table 1, together with standard deviations to show the difficulty of measuring such low concentrations in the sludge matrix. Chosen substances are two of the ones mentioned in the water frame work directive proposal (Diclofenac and Ehinylestradiol), substances that are heavily deviating from other reported values in literature (Fick, 2011; Wahlberg, 2010) (Bupropion, Ciprofloxacin and Miconazole), substances occurring in highest concentrations (Ciprofloxacin, Dipyridamol, Sertraline, Irbesartan and Ketoconazole) and substances that seem to be reduced during anaerobic digestion (Irbesartan and Trimethoprim).

Table 1- Average values and standard deviation (in italic) of chosen pharmaceutical substances in sludges (untreated, digested and pasteurized);unit: ng/g TS. Digested sludges with 24 hours exposure time. N=2 for all samples except 70°C, 60 min, where N=3. The total solids (TS) content indicated for each sludge was reduced by ~60% by anaerobic digestion.

Substance	Untreated (4.9 % TS)		35°C, 15 days HRT (2.0 % TS)		55°C, 15 days HRT (2.1 % TS)		60°C, 15 days HRT (2.3 % TS)		70°C, 60 min (4.9 % TS)	
Diclofenac*	<10	-	<10	-	<10	-	<10	-	<10	-
Ethinylestradiol*	<10	-	<10	-	<10	-	<10	-	<10	-
Bupropion	5	*1*	13	*6*	52	*10*	13	*3*	5	*3*
Ciprofloxacin	2 100	*800*	4 100	*600*	1 500	*30*	1 400	*500*	2 400	*570*
Miconazole	36	-	19	*13*	50	*30*	10	-	10	*3*
Ciprofloxacin	2 100	*800*	4 100	*600*	1 500	*40*	1 400	*500*	2 400	*570*
Dipyridamol	190	*130*	490	*30*	470	*270*	320	*10*	190	*30*
Sertraline	280	*10*	670	*40*	810	*250*	180	*110*	340	*110*
Irbesartan	1 900	*2 600*	540	*380*	20	*20*	60	*10*	240	*100*
Ketoconazole	200	*60*	160	*50*	220	*90*	90	*0*	110	-
Irbesartan	1 900	*2 600*	540	*380*	20	*20*	60	*10*	240	*100*
Trimethoprim	19	*21*	<0.1	-	1	*0*	5	*0*	30	*20*

* Limit of quantification 10 ng/g TS

In general no reduction of pharmaceuticals could be observed at any temperature or minimum exposure time, except for Irbesartan and Trimethoprim. Irbesartan (blood pressure lowering substance) was seen to be reduced by pasteurization or anaerobic digestion (Table 1) with higher reduction for thermophilic digestion. Trimethoprim (antibiotic substance) was reduced by anaerobic digestion but not by pasteurization. The concentrations of pharmaceuticals in the mesophilic sludge agreed well with results in recent studies on Swedish sludges (Wahlberg et al., 2010; Fick et al., 2011). Some substances seem to increase in concentration during digestion. This indicates a high uncertainty of the method resulting in high standard deviations or that the substances actually occur in higher concentrations after digestion. The uncertainty in the method is a probable reason, because in 70% of the cases with increasing concentrations after digestion, the standard deviations are really high for both concentrations in untreated and digested sludge. If the substances actually occur in higher concentrations after digestion there are two possibilities: The digestion may lead to extraction of pharmaceuticals from the sludge by reduction of the particles on which the pharmaceuticals can adsorb. The other reason could be that degradation products, that has been released in

conjugated form after metabolization in the human body and then ended up in wastewater entering the WWTP, can be recreated to the mother substance. This phenomenon has been discussed for wastewater in other studies (Naturvårdsverket, 2008; Wahlberg et al., 2010)
High standard deviations (13-118%) for the results were seen both for samples analysed in duplicate and triplicate and are explained by the difficulties of making analyses of the sludge matrix. Recovery tests performed by the same lab as in the study on both wastewater and sludges showed in general lower degrees of recovery and higher standard deviations for sludge. More reliable analysis methods for sludge are consequently needed.

Polycyclic aromatic hydrocarbons
Samples were analysed in duplicates and certified reference materials in triplicates, yielding relative standard deviations (RSD) of 2-17%, and recoveries of 98-112% (benzo[g,h,i]perylene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]perylene, and naphthalene, i.e., the lightest and the heaviest PAHs had the same excellent RSD 5-18% and, as expected, lower recoveries of 25-32%).

Out of the 15 PAH analysed, 11 were detected and quantified. Fluoranthene (0.15-0.66 mg/kg TS), phenanthrene (0.10-0.49) and pyrene (0.13-0.56) were the 3 PAHs detected in all samples. Anthracene, benzo[a]anthracene and fluorene were present in only a few samples above the quantification limi (1, 3 and 8 samples out of 28 analysed). Benzo[b]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]perylene were also detected above the detection limits. The summary concentrations of the 8 PAHs (anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, fluoranthene, indeno[1,2,3-cd]perylene and naphthalene) included in the proposal for the new WFD (EC, 2012) are found in Table 2.

Table 2. Summary concentrations of PAH-7* mentioned in the WFD proposal (2012) in the sludges after different treatments/digestion. Results in mg/kg TS.

Minimum exposure time (h)	Untreated	70°C, 60 min	35°C, 15 days HRT	55°C, 15 days HRT	60°C, 15 days HRT	55°C, 7 days HRT	60°C, 7 days HRT
24	1.7	1.8	2.3	2.3	2.5	2.3	2.8
6	1.6	1.6	2.4	2.3	2.5	2.3	2.3
2.5	1.6	1.7	2.5	2.2	2.4	2.3	2.4
2	1.4	1.7	2.4	2.1	2.2	2.1	2.2

*benzo[k]fluoranthene was not measured, but dibenzo[a,h]anthracene was included

The results show that no statistical significant reduction of the sum of PAH was achieved during digestion or pasteurization, a result that is also valid for several of the individual PAHs in the study. For single PAHs reduction up to 60% during digestion was seen, namely, for indeno[1,2,3-cd]pyrene and dibenzo[a,h]antracene (analysed as sum) and for benzo[g,h,i]perylene, see Figure 1. Digestion at 35 and 55°C resulted in similar reduction of these 3 PAHs while digestion at 60°C resulted in lower reduction. No statistical correlation between reduction and exposure time was seen. No reduction of the 3 PAHs was seen during pasteurization. The total concentration of sum PAH (1.4-2.8 mg/kg TS) was high compared to the results found in Olofsson et al. (2013), where sludge from seven Swedish WWTP:s were analysed (ranging from 0.47-1.7 mg/kg TS).

Reduction of the same 3 heavy PAH was also seen in a study by Trably et al. (2003) but in that study was also seen reduction of other PAHs which could be explained by the long HRT

(41 days) used in their digestion tests, since a long retention time may lead to an increased degradation. However, no significant difference in reduction between the different HRT:s (7 days and 15 days) applied in this study was seen. In Christensen et al. (2004) naphthalene was reduced during digestion with much longer exposure time than in this study. Higher reduction at longer exposure times could be explained by a favouring of PAH reduction when easily degradable matter already has been consumed. This reasoning is supported by the results in Trably et al. (2003) which showed that the PAH reduction increased when the biogas production decreased. Alternatively the decrease in gas production could be a result of inhibition of methanogens from the increase of hydrogen produced during degradation of PAH shown by Christensen et al. (2004). The latter is more unlikely since the hydrogen production from reduction of PAH should be insignificant compared to the other hydrogen produced in the biogas process.

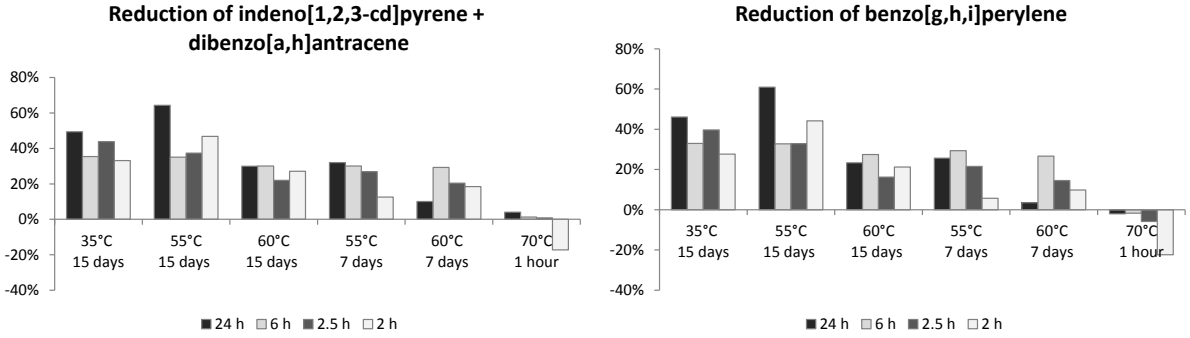

Figure 1. Reduction of 3 PAH (indeno[1,2,3-cd]pyrene and dibenzo[a,h]antracene (analysed as group) and benzo[g,h,i]perylene) during digestion and pasteurization.

The influence of digestion temperature on reduction is contradictory to other studies. The results for the 3 PAH that were reduced showed that the reduction was lower at 60°C than at 35°C or 55°C. Christensen et al. (2004) and Trably et al. (2003) state that the reduction is increased with an increased temperature and with an adjusted microbial population. A possible explanation to the lower reduction seen at 60°C could be a sub-optimal microbial population of the anaerobic digestion metabolism deriving from the higher temperature of digestion. Such a hypothesis is supported by the fact that methane production was 10% less in reactors operated at 60°C than at 55°C (Kjerstadius et al., 2013). This effect was contributed to the shift in methanogenic genera from methanosarcina to methansaeta which occurs around 60-62°C (Van Lier et al., 1993; Zinder, 1990; Zehnder, 1988). However since the complete degradation pathway of PAH in anaerobic digesters with sludge as feed was unknown a sup-optimal microbial population remains a hypothesis.

CONCLUSIONS

The potential for improving the sludge quality by reduction of harmful contaminants during anaerobic digestion or pasteurization at 70°C seems to be low.

Pharmaceutical residues are generally not reduced during digestion at any investigated temperature (35, 55 and 60°C) or by pasteurization (70°C for 1 hour). In individual cases, some reduction occurs but this cannot be determined due to the low accuracy when analysing sludge samples. PAHs were generally not reduced during digestion or pasteurization, but were reduced to some extent (up to 60%) during digestion in three individual cases (indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene (analysed as sum) and benzo[g,h,i]perylene). Digestion

at 35 and 55°C resulted in about the same order of reduction of the three individual PAHs which was higher than for digestion at 60°C.

ACKNOWLEDGEMENTS
The Swedish Water & Wastewater Association, SWWA is thanked for the funding of the project and VA SYD, the municipal joint authority of water and sewage departments of southwest Sweden is thanked for assistance with and provision of digestion equipment. Technicians Christina Maj Hagberg and Sinh Hy Nguyen are acknowledged.

REFERENCES
Clarke, B., Porter, N., Symons, R., Blackbeard, J., Ades, P. & Marriott, P. 2008 Dioxin-like compounds in Australian sewage – Review and national survey. *Chemosphere*, **72(**8), pp. 1215-1228.

Clarke, B., Porter, N., Symons, Marriott, P. Stevenson, G.J. & Blackbeard, J. 2010 Investigating the distribution of polybrominated diphenyl ethers through an Australian wastewater treatment plant. *Science of the total environment*, **408(7**), pp.1604-1611.

Christensen, N., Batstone, D.J., He, Z., Angelidaki, I. & Schmidt, J.E. 2004 Removal of polycyclic aromatic hydrocarbons (PAHs) from sewage sludge by anaerobic degradation. *Water Science and Technology*, **50**(9), pp. 237–244.

EC (European Commission) 2012 Proposal for a directive of the European parliament and of the council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Brussels: European Commission.

Erhardt, W. & Prüeß, A. 2001 Organic contaminants in sewage sludge for agricultural use. Report for the European Commission, Joint Research Centre.

Fick, J., Lindberg, R.H., Kaj, L. & Brorström-Lundén, E. 2011 Results from the Swedish National Screening programme 2010 – Subreport 3. Pharmaceuticals. Stockholm: Swedish Environmental Research Institute (IVL).

Grabic, G., Fick, J., Lindberg, R.H., Fedorova, G. & Tysklind, M. 2012 Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry. *Talanta*, **100**, pp 183-195.

Hey, G., Ledin, A., la Cour Jansen, J. & Andersen, H.R. 2012 Removal of pharmaceuticals in biologically treated wastewater by chlorine dioxide or peracetic acid. *Environmental Technology*, **33**(9), pp. 1041-1047.

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., La Cour Jansen, J. & Andersen, H.R. 2011 Determination of sorption of seventy-five pharmaceuticals in sewage sludge, *Water Research*, **45**, pp. 4470-4482.

Kjerstadius, H., la Cour Jansen, J., Haghighatafshar, S. & Davidsson, Å. 2013 Hygienization of sludge through anaerobic digestion at 35, 55 and 60 ºC. Proceedings of the 1st International IWA Conference on Holistic Sludge Management, Västerås, Sweden 2013.

Naturvårdsverket 2003 Organic contaminants in sewage sludge. Rapport 5217. Stockholm: Naturvårdsverket.

Naturvårdsverket 2008 Rapport 5794: Avloppsreningsverkens förmåga att ta hand om läkemedelsrester och andra farliga ämnen (The ability of municipal waste water treatment plants to handle pharmaceutical residues and other harmful substances). Stockholm: Naturvårdsverket.

Olofsson, U., Brorström-Lundén, E., Kylin, H. & Haglund, P. 2013 Comprehensive mass flow analysis of Swedish sludge contaminants. *Chemosphere*, **90**(1), pp 28-35.

Trably, E., Patureau, D. & Delgenes, J.P. 2003 Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. *Water Science & Techn*ology, **48 (**4), pp. 53–60.

Wahlberg, C., Björlenius, B. & Paxéus, N. 2010 Läkemedelsrester i Stockholms vattenmiljö. Stockholm: Stockholm Vatten.

Van Lier, J.B., Hulsbeek, J.J.M., Stams, A. & Lettinga, G. 1993 Temperature Susceptibility of thermophilic sludge: Implications for reactor start-up and operation. *Bioresource Technology*, **43**, 227-235.

Zehnder, J.B.A. (red.) 1988 Biology of anaerobic microorganisms. New York: John Wiley & sons.

Zinder, S.H. 1990 Conversion of acetic acid to methane by thermophiles. New York : *FEMS Microbiology reviews*, **75**, 125-138.

Økland, T.E., Wilhelmsen, E. & Solevåg, Ø. 2005 A study of the priority substances of the Water Framework Directive. Report: Oslo, Bergfald & Co.